STIC-ILL

V. NO

From: Sullivan, Daniel
Sent: Monday, December 30, 2002 4:27 PM
To: STIC-ILL
Subject: Request

425794

Please send the following:

AUTHOR: Binder S.W.; Said J.W.; Shintaku I.P.; Pinkus G.S.
SOURCE: American Journal of Clinical Pathology, (1992) 97/6 (759-763)

PubMed ID: 3524693
AUTHOR: Carrere J; Figarella C; Guy O; Thouvenot J P
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1986 Aug 6) 883 (1) 46-53

AUTHOR(S): Gundlach, H. Gerd
SOURCE: Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie (1970), 351(6), 696-700

AUTHOR(S): Specchia, G.; Petroboni, V.; Fratino, P.; Dander, B.
SOURCE: Bollettino - Societa Italiana di Biologia Sperimentale (1970), 46(3), 111-14

AUTHOR(S): BONIN A; ROY C C; LASALLE R; WEBER A; MORIN C L
SOURCE: J PEDIATR, (1973) 83 (4), 594-600

AUTHOR(S): LESI C; D'ERIL G V M; ZONI L; MALAGUTI P
SOURCE: 16TH MEETING OF THE EUROPEAN PANCREATIC CLUB, CASCAIS, PORTUGAL, SEPT. 13-15, 1984. DIGESTION, (1984) 30 (2), 114-115

Thank you.

Daniel M. Sullivan
Examiner AU 1636
Room: 12D12
Mail Box: 11E12
Tel: 703-305-4448

09856319

9156118

Volume 83
Number 4

Fecal chymotrypsin: A reliable index of exocrine pancreatic function in children

The 72 hour fecal output of chymotrypsin expressed in milligrams per kilogram of body weight was measured in 156 children. Values in 35 control subjects and in 56 children with various intestinal and hepatobiliary diseases did not overlap with those of 53 children with cystic fibrosis or with three children who had chronic pancreatic disease with steatorrhea. However, in one child with chronic relapsing pancreatitis and in seven with cystic fibrosis who had a normal fat excretion, enzyme activity was normal. The only value within the range associated with pancreatic insufficiency was seen in a case of intestinal scleroderma. Duodenal enzyme concentrations in 35 children correlated well with fecal measurements in primary pancreatic disease with a significant degree of achylia.

André Bonin,* Claude C. Roy, Roger Lasalle, Andrée Weber, and Claude L. Morin, *Montreal, Quebec, Canada*

THE DIAGNOSIS of pancreatic exocrine insufficiency is facilitated by the clinical history and by various laboratory studies, but it should be confirmed by exploration of the functional capacity of the pancreas.

Until Haverback and associates[1] described a method for determining trypsin and chymotrypsin in feces, the diagnosis of pancreatic achylia rested entirely on the analysis of duodenal contents. Duodenal drainage in the pediatric age group is time consuming, uncomfortable for the young child, and presents difficulties. To be reliable, the total duodenal secretion, free of gastric juice, must be collected over specified time periods and analyzed for volume, bicarbonate content, and enzymatic activity before and after pancreatic stimulation with secretin and pancreozymin.[2]

The value of stool chymotrypsin for the diagnosis of pancreatic achylia has already been documented.[1, 3-10] However, a high incidence of low values was found in adult patients with steatorrhea of nonpancreatogenous origin.[1, 3, 5, 10] Furthermore, studies in children have been largely limited to patients

From the Department of Pediatrics, Hôpital Sainte-Justine, and the University of Montreal.

Supported by the Canadian Cystic Fibrosis Foundation.

Presented in part to the Cystic Fibrosis Meeting, Atlantic City, N. J., May, 1970, and to the Thirteenth International Congress of Pediatrics, Vienna, August, 1971.

**Address: Hôpital Ste-Justine, 3175 Ste. Catherine Rd., Montreal 250, Quebec, Canada.*

Vol. 83, No. 4, pp. 594-600

with cystic fibrosis, and
made to correlate resu
in duodenal juice. T
with a large group of c
a variety of pancreatic,
disorders, shows that f
pressed as milligrams p
gram of body weight
index of exocrine panc

MATERIAL AND

Sixty-four children w
were studied. The age
60 patients with cystic
years. In the 40 patie
in the Cystic Fibrosis
were carried out on a
least five days after dis
and pancreatic enzyme
dren with cystic fibro
during the course of an
up and had never recei
apy for their disease. C
noncystic fibrosis panc
had chronic relapsing
had exocrine pancrea
bone marrow dysfunctio

Also studied were 57
hepatic and intestinal
negative sweat tests and
of pancreatic disease.
± S.D.) of the 20 subje
was 0.9 ± 0.9 year. The
hepatic biliary atresia,
intrahepatic bile ducts,
cirrhosis, and 5 with ne
13 patients with gluten
had a mean age of 3.5
children aged 2.7 ± 3.7
under the category of c
out fat malabsorption:
colon syndrome,[11, 12] and
isomaltase deficiency, se
with intermittent wa
chronic diarrhea of unk
were 3 children with in
early infancy,[13] 3 with
drome following ileal r
the stagnant loop syndr
testinal scleroderma.

with cystic fibrosis, and no attempt has been made to correlate results in feces with those in duodenal juice. This report, concerned with a large group of children suffering from a variety of pancreatic, hepatic, and intestinal disorders, shows that fecal chymotrypsin expressed as milligrams per 72 hours per kilogram of body weight constitutes a reliable index of exocrine pancreatic function.

MATERIAL AND METHODS

Sixty-four children with pancreatic disease were studied. The age (mean ± S.D.) of the 60 patients with cystic fibrosis was 4.0 ± 3.3 years. In the 40 patients regularly followed in the Cystic Fibrosis Clinic, investigations were carried out on an outpatient basis at least five days after discontinuing antibiotics and pancreatic enzymes. The other 20 children with cystic fibrosis were investigated during the course of an initial hospital work-up and had never received any form of therapy for their disease. Of four children with noncystic fibrosis pancreatic disease, three had chronic relapsing pancreatitis and one had exocrine pancreatic insufficiency and bone marrow dysfunction.[11]

Also studied were 57 children with various hepatic and intestinal disorders who had negative sweat tests and no clinical evidence of pancreatic disease. The average age ($\bar{X}$ ± S.D.) of the 20 subjects with liver disease was 0.9 ± 0.9 year. There were 9 with extrahepatic biliary atresia, 1 with "paucity of intrahepatic bile ducts," 5 with postnecrotic cirrhosis, and 5 with neonatal hepatitis. The 13 patients with gluten-induced enteropathy had a mean age of 3.5 ± 4.1 years. Sixteen children aged 2.7 ± 3.7 years were grouped under the category of chronic diarrhea without fat malabsorption: 13 had the "irritable colon syndrome,"[12] and one each had sucrase-isomaltase deficiency, selective IgA deficiency with intermittent watery diarrhea, and chronic diarrhea of unknown etiology. There were 3 children with intractable diarrhea of early infancy,[13] 3 with the short bowel syndrome following ileal resection, and 2 with the stagnant loop syndrome secondary to intestinal scleroderma.

The control population was made up of 20 healthy children who were evaluated in the outpatient clinic and 15 children admitted to the hospital without any evidence of disease of the gastrointestinal tract, liver, or pancreas. The mean age of these children was 4.6 ± 3.5 years. None had a family history of cystic fibrosis.

Stools were collected between charcoal markers given 72 hours apart. The feces were kept frozen during and after completion of the collections. Assays for fat and chymotrypsin were usually carried out within one week. Fat determinations were done using the method of Van de Kamer and associates[14] or the method of Jeejeebhoy and associates[15] in seven children with hepatic disorders who were on a low-fat diet supplemented with medium chain triglycerides. Fecal chymotrypsin determinations were carried out on duplicate aliquots of homogenized stool diluted with water. Duplicates agreed within ± 2 per cent and results were expressed in milligrams per 72 hour stool collection per kilogram of body weight.

In order to establish a correlation between fecal and duodenal chymotrypsin, duodenal intubation was carried out in 35 patients and 7 control subjects. After the tip of a weighted polyethylene tube was placed in the third portion of the duodenum under fluoroscopic control, gastric suction was carried out through a nasogastric catheter. Duodenal contents were collected for a single period of 20 minutes following the intravenous administration of cholecystokinin-pancreozymin (Boots Pure Drug, Nottingham, England) at a dose of 1.5 unit per kilogram. The pH of the duodenal aspirates, collected in tubes placed in Dry-Ice, varied between 6.0 and 8.3. Duplicate aliquots were assayed for chymotrypsin and results were expressed in micrograms per milliliter of duodenal juice. The method of Haverback and associates[1] was used for both stool and duodenal chymotrypsin.

RESULTS

The children with cystic fibrosis were divided according to the values obtained for

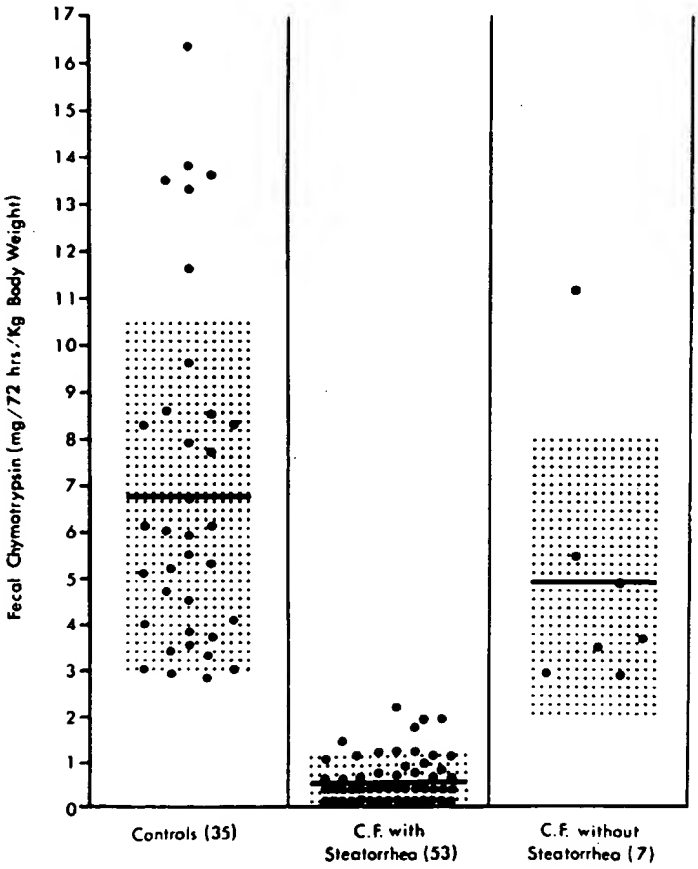


Fig. 1. Fecal chymotrypsin output (mg./72 hours/Kg. body weight) in 35 control subjects, 53 children with cystic fibrosis *(C.F.)* and steatorrhea, and in 7 children with cystic fibrosis without fat malabsorption (<4.5 Gm./24 hours). The horizontal lines represent average values and the shaded areas 1 S.D.

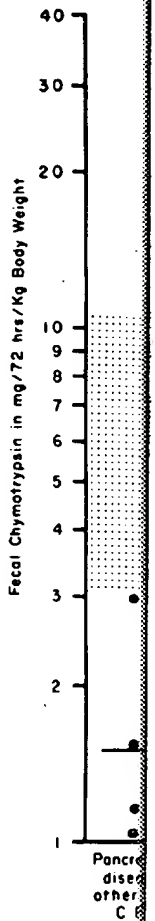


Fig. 2. Fecal chymot
and pancreatic diso
averages for the va
control subjects.

the 24 hour stool fat excretion. There were 53 with a mean excretion of 22.7 Gm. and 7 with less than 4.5 Gm. per 24 hours, the upper limit of normal in our laboratory. The average value for the control subjects was 2.6 Gm. It is apparent in Fig. 1 that fecal chymotrypsin values expressed in milligrams per 72 hours per kilogram of body weight clearly separated the cystic fibrosis patients with steatorrhea from both the control subjects and children with cystic fibrosis without fat malabsorption.

Results in the 57 children with hepatic or intestinal disorders and in the 4 with pancreatic disease other than cystic fibrosis are shown in Fig. 2. Three of the latter did not have significant pancreatic insufficiency to cause fat malabsorption. Nevertheless, the fecal chymotrypsin in this group was statistically lower ($P < 0.01$) than that of the control population. On the other hand, values in hepatic disorders, gluten-induced enteropathy, chronic diarrhea without fat malabsorption, intractable diarrhea, and the short bowel syndrome did not differ from those in control subjects.

Transit time, estimated by the number of hours taken by the charcoal marker given 72 hours apart to begin and end stool collections, averaged seven hours in the three infants with ileal resections who had the highest mean output of fecal chymotrypsin (23.4 mg.). The two children with the stagnant loop syndrome had a mean chymotrypsin output (2.6 mg.) within t
sociated with pancreati
time averaged 49 hours.

Fig. 3 is a plot of the
tions of chymotrypsin
venous administration o
creozymin and the cor
motrypsin output in 7 c
35 patients. A concent
milliliter was the lowe
control subjects and w
limit of normal. This v
low the figure of 250 μ
was the lowest concent
adult control subjects

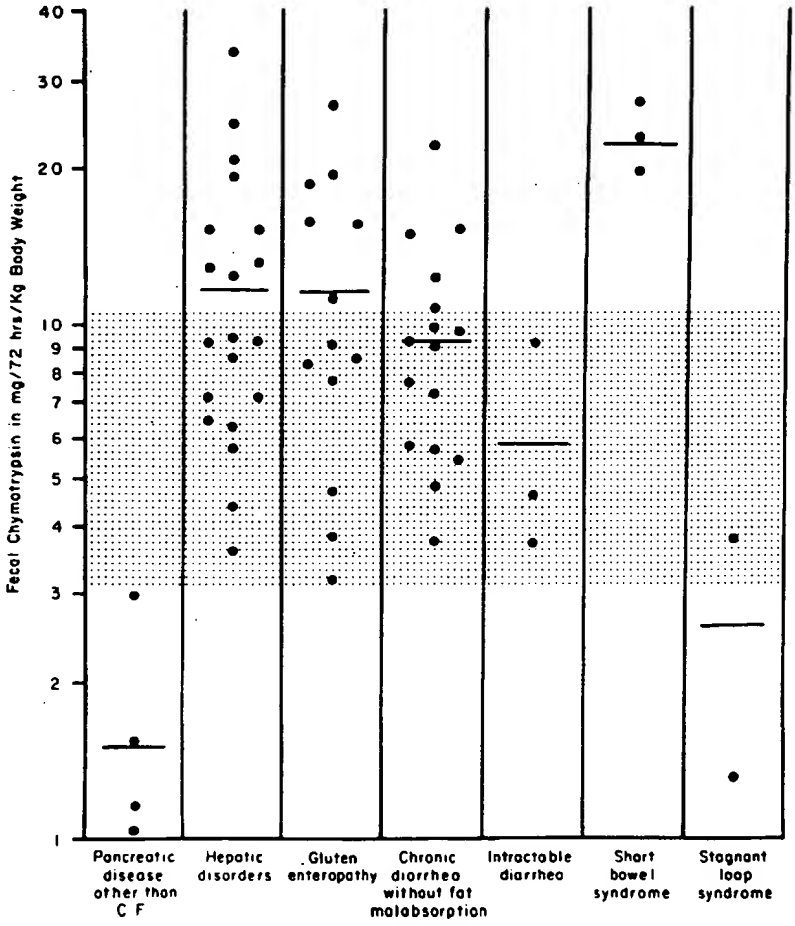


Fig. 2. Fecal chymotrypsin output (mg./72 hours/Kg. body weight) in gastrointestinal, hepatic and pancreatic disorders other than cystic fibrosis *(C.F.)*. The horizontal lines represent the averages for the various groups. The shaded area corresponds to the mean ± 1 S.D. of the control subjects.

put (2.6 mg.) within the range of values associated with pancreatic disease; their transit time averaged 49 hours.

Fig. 3 is a plot of the duodenal concentrations of chymotrypsin following the intravenous administration of cholecystokinin-pancreozymin and the corresponding fecal chymotrypsin output in 7 control subjects and in 35 patients. A concentration of 200 μg per milliliter was the lowest value obtained in control subjects and was taken as the lower limit of normal. This value is somewhat below the figure of 250 μg per milliliter which was the lowest concentration obtained in 40 adult control subjects by Ammann and associates[9] after secretin and pancreozymin. The only patient with pancreatic disease who had a measurable concentration of duodenal chymotrypsin had a fecal chymotrypsin output at the lower limit of normal, and steatorrhea could not be documented. Chronic diarrhea without fat malabsorption was associated with the recovery of normal duodenal and fecal chymotrypsin. Two patients with gluten-induced enteropathy had abnormal duodenal chymotrypsin concentrations; the value of 135 μg per milliliter was obtained in a severely malnourished 1-year-old child. Marasmus was also present in the infant with "intractable diarrhea" who had a concentra-

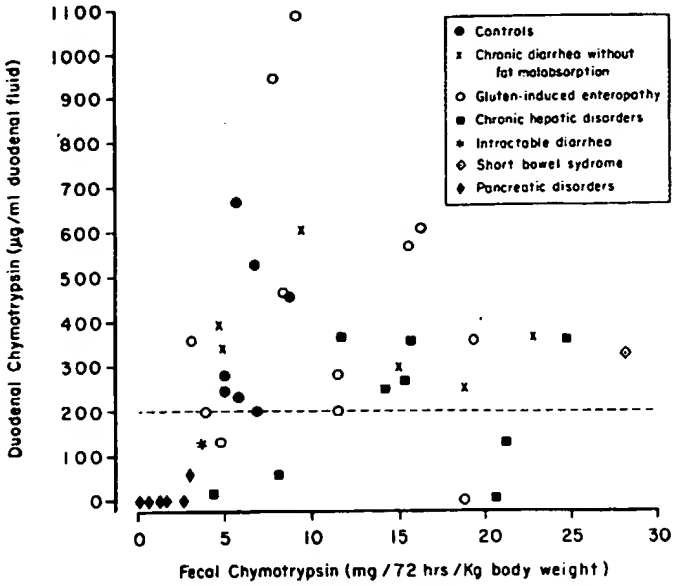


Fig. 3. Plot of duodenal chymotrypsin concentrations (μg/ml.) versus fecal chymotrypsin output (mg./72 hours/Kg. body weight). Dotted horizontal line indicates lowest duodenal value (200 μg/ml.) obtained in control subjects.

tion of 134 μg per milliliter. Abnormally low duodenal chymotrypsin was documented in four patients with liver disease; their fecal chymotrypsin output was normal.

DISCUSSION

Fecal chymotrypsin cannot be considered a diagnostic test in cystic fibrosis since about 20 per cent of patients with cystic fibrosis have no evidence of pancreatic insufficiency[16] or only partial pancreatic achylia.[2] In fact, the output of chymotrypsin in seven cases of cystic fibrosis without fat malabsorption did not differ from the output measured in control subjects. However, the simple quantitation of chymotrypsin in a 72 hour collection of stools provided a satisfactory means of separating 53 children with cystic fibrosis and with pancreatic insufficiency manifested by steatorrhea from seven children with cystic fibrosis and with normal exocrine function of the pancreas.

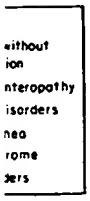
The measurement in 57 children with hepatic and intestinal disorders has yielded only one value (1.3 mg.) within the range observed in pancreatic disorders. The good discriminant value achieved in the present study contrasts with the high incidence in the reduction of stool chymotrypsin reported in adults with nonpancreatic disorders.[1, 3, 5, 10] One report found reduced activity in 25 per cent of cases.[5] It is likely that quantitation of fecal chymotrypsin in a 72 hour stool specimen represents a better general index of the status of the exocrine pancreas[17] because measurement of activity in a random stool specimen disregards variations in enzyme output related to periods of pancreatic stimulation and quiescence. The 72 hour stool weights varied between 65 and 775 Gm. in the 156 subjects of the present report. Therefore, had random specimens been used, a number of those with steatorrhea and a large fecal mass would have had subnormal chymotrypsin values on the basis of dilution. The wide age range (1 month to 16 years) prompted the reporting of 72 hour values in milligrams per kilogram of body weight.

As mentioned previously,[1] the activity of fecal enzyme depends not only on the output of pancreatic enzyme but also on the inactivation which might take place during intestinal passage. The stability of chymotrypsin is not only influenced by motility but also by

a host of other facto
degrees of binding
testinal debris.[18] Ra
creases fecal enzym
enzyme inactivation
interesting to note th
tween chymotrypsin
three cases of short
the two with the sta

There is only one
ing the degree of pa
by the secretin-panc
chymotrypsin.[9] In
moderate pancreatic
dence of falsely high
ues was 5 per cent
tively.[9] In the pres
good correlation bet
measurements in th
pancreatic disease
The fecal value of
child with chronic r
normal fat absorptio
concentration of on
confirms the limited
tients with pancrea
degree of achylia.

Interpretation of
ance with abnormal
tubations in two pa
opathy, in four with
in the young infant
rhea" is more difficu
denal intubation use
permit complete co
cretions free of gas
ability of chymotryps
known[19] and may e
activity found in 7
bation studies. It is
nutrition, a striking
with gluten enterop
with intractable dia
alterations in pancre
the output of pancre
to amino acids is re
tropical sprue, it is
kinin-pancreozymin.
trypsin values found
intrahepatic biliary



30

it)

al chymotrypsin out-
owest duodenal value

the high incidence in the
ymotrypsin reported in
creatic disorders.[1, 3, 5, 10]
duced activity in 25 per
likely that quantitation
in in a 72 hour stool
a better general index
exocrine pancreas[17] be-
of activity in a random
gards variations in en-
to periods of pancreatic
iescence. The 72 hour
etween 65 and 775 Gm.
of the present report.
om specimens been used,
with steatorrhea and a
ld have had subnormal
on the basis of dilution.
(1 month to 16 years)
ing of 72 hour values in
am of body weight.
viously,[1] the activity of
s not only on the output
e but also on the inacti-
take place during in-
stability of chymotrypsin
d by motility but also by

a host of other factors which lead to varying degrees of binding to mucosal cells and intestinal debris.[18] Rapid intestinal transit increases fecal enzyme activity by diminished enzyme inactivation.[9] In this regard, it is interesting to note the inverse relationship between chymotrypsin and transit time in the three cases of short bowel syndrome and in the two with the stagnant loop syndrome.

There is only one report in adults correlating the degree of pancreatic achylia revealed by the secretin-pancreozymin test with fecal chymotrypsin.[9] In patients with severe and moderate pancreatic insufficiency, the incidence of falsely high fecal chymotrypsin values was 5 per cent and 16 per cent, respectively.[9] In the present study, there was a good correlation between duodenal and fecal measurements in the five cases of primary pancreatic disease with fat malabsorption. The fecal value of 3.0 mg. in a 9-year-old child with chronic relapsing pancreatitis and normal fat absorption, who had a duodenal concentration of only 63 μg per milliliter, confirms the limited value of the test in patients with pancreatic disease with a mild degree of achylia.

Interpretation of results in feces at variance with abnormal values in duodenal intubations in two patients with gluten enteropathy, in four with chronic liver disease, and in the young infant with "intractable diarrhea" is more difficult. The technique of duodenal intubation used in this study does not permit complete collection of duodenal secretions free of gastric juice. The vulnerability of chymotrypsin to gastric juice is well known[19] and may explain the low duodenal activity found in 7 out of 42 duodenal intubation studies. It is also possible that malnutrition, a striking clinical feature in a case with gluten enteropathy and in the infant with intractable diarrhea could have led to alterations in pancreatic function.[20] Although the output of pancreatic enzymes in response to amino acids is reduced in adults with nontropical sprue, it is normal after cholecystokinin-pancreozymin.[21] The low duodenal trypsin values found in four children with intrahepatic biliary atresia were explained by incomplete activation of the zymogen by enterokinase in the absence of bile acids.[22] However, other enzymes were normal. Evaluation of the reliability of fecal chymotrypsin for the assessment of the more discrete alterations in pancreatic function expected in nonpancreatic disorders must await more complete duodenal studies using a more sophisticated technique such as isolation of the duodenal loop with a triple lumen tube.[2]

CONCLUSIONS

A fecal chymotrypsin output of more than 3 mg. per 72 hours per kilogram of body weight essentially rules out primary pancreatic disease in children. However, in cases where the degree of pancreatic insufficiency does not lead to steatorrhea, low normal values may be anticipated. In the investigation of the child with malabsorption, it is advocated as a screening test. However, in certain cases this should be supplemented by the more discriminant analysis of function provided by duodenal studies.

REFERENCES

1. Haverback, B. J., Dyce, B. J., Gutentag, P. J., and Montgomery, D. W.: Measurement of trypsin and chymotrypsin in stool: A diagnostic test for pancreatic exocrine insufficiency, Gastroenterology 44: 588, 1963.
2. Hadorn, B., Zoppi, G., Shmerling, D. H., Prader, A., McIntyre, I., and Anderson, C. M.: Quantitative assessment of exocrine pancreatic function in infants and children, J. Pediatr. 73: 39, 1968.
3. Dyck, W., and Ammann, R.: Quantitative determination of fecal chymotrypsin as a screening test for pancreatic exocrine insufficiency, Am. J. Dig. Dis. 10: 530, 1965.
4. Banwell, J. G., Leonard, P. J., and Lobo, R. M. F.: Measurement of trypsin and chymotrypsin activity in stools to detect chronic pancreatic disease, Gut 6: 143, 1965.
5. Wilding, P., Banwell, J. G., and Cooke, W. T.: Fecal enzyme assay as an aid to the diagnosis of pancreatic enzyme insufficiency, Am. J. Dig. Dis. 11: 722, 1966.
6. Barbero, G. J., Sibinga, M. S., Marino, J. M., and Seibel, R.: Stool trypsin and chymotrypsin, Am. J. Dis. Child. 112: 536, 1966.
7. Mullinger, M., and Palasi, M.: Tryptic and chymotryptic activity of stools of newborn infants, Pediatrics 38: 657, 1966.
8. Ammann, R., and Kashiwagi, H.: Pancreatic exocrine insufficiency and proteolytic enzymes in stool: A critical evaluation of a new diag-

nostic test in various forms of steatorrhea, Helv. Med. Acta 33: 220, 1966.
9. Ammann, R. W., Tagwecher, E., Kashiwagim, H., and Rosenmund, H.: Diagnostic value of fecal chymotrypsin and trypsin assessment for detection of pancreatic disease, Am. J. Dig. Dis. 13: 123, 1968.
10. Smith, J. S., Ediss, I., Mullinger, M. A., and Bogoch, A.: Fecal chymotrypsin and trypsin determinations, Can. Med. Assoc. J. 104: 691, 1971.
11. Shwachman, H., Diamond, L. K., Oski, F. A., and Khaw, K.-T: The syndrome of pancreatic insufficiency and bone marrow dysfunction, J. PEDIATR. 65: 645, 1964.
12. Davidson, M., and Wasserman, R.: The irritable colon of childhood, J. PEDIATR. 69: 1027, 1966.
13. Hyman, C. J., Reiter, J., Rodnan, J., and Drash, A. L.: Parenteral and oral alimentation in the treatment of the nonspecific protracted diarrheal syndrome of infancy, J. PEDIATR. 78: 17, 1971.
14. Van de Kamer, J. H., ten Bokkel Huininck, H., and Wejers, H. A.: Rapid method for the determination of fat in feces, J. Biol. Chem. 177: 347, 1949.
15. Jeejeebhoy, K. N., Ahmad, S., and Kozak, G.: Determination of fecal fats containing both medium and long chain triglycerides and fatty acids, Clin. Biochem. 3: 157, 1970.
16. Shwachman, H., Dooley, R. R., Mass, C., and Evans, A. C.: Cystic fibrosis of the pancreas without pancreatic enzyme deficiency, Am. J. Dis. Child. 92: 520, 1956.
17. Adham, N. F., Dyce, B. J., Geokas, M. C., and Haverback, B. J.: Stool chymotrypsin and trypsin determinations, Am. J. Dig. Dis. 12: 1272, 1967.
18. Goldberg, D. M., Campbell, R., and Roy, A. D.: Binding of trypsin and chymotrypsin in the human small intestine, Biochim. Biophys. Acta 167: 613, 1968.
19. Goldberg, D. M., Campbell, R., and Roy, A. D.: Fate of trypsin and chymotrypsin in the human small intestine, Gut 10: 477, 1969.
20. Barbezat, G. O., and Hansen, J. D. L.: The exocrine pancreas and protein-calorie malnutrition, Pediatrics 42: 77, 1968.
21. Di Magno, E. P., Go, V. L. W., and Summerskill, W. H. J.: Impaired cholecystokinin-pancreozymin secretion, intraluminal dilution, and maldigestion of fat in sprue, Gastroenterology 63: 25, 1972.
22. Hadorn, B., Troesch, V., Götze, H., and Bender, S. W.: Disturbance of trypsinogen activation in children with intrahepatic biliary atresia, European Society for Pediatric Gastroenterology Meeting, 1972.

Tear so
at vario
fibrosis
without

When compared to
in this study had:
flow rates but the
and the same mini
ocular bacterial in
punctate fluoresce
whom had signs or
with vitamin A de

Stella Y. Botelh
Mary L. Rosen

ALTHOUGH
secretions from s
glands, there is so
these orbital glan

From the Departm
Ophthalmology, a
of Pennsylvania S

Supported in part
National Cystic Fi
and from United
National Institute
Institute No. 5 R0

Presented in part
and October, 197
Meetings of the A
in Vision and Op

Reprint address: Dep
of Pennsylvania Schoo
19174.